Susceptibility of Nosocomial *Staphylococcus aureus* to Chlorhexidine after Implementation of a Hospital-wide Antiseptic Bathing Regimen

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Abstract

Background: Bathing hospitalized patients with chlorhexidine gluconate is an effective way to reduce healthcare-associated infections by multi-drug-resistant organisms such as MRSA. There is concern that widespread use of chlorhexidine (CHX) will promote the emergence of bacterial resistance. This study assessed CHX susceptibility of bloodstream-infecting *S. aureus* from four distinct time periods with well-characterized usage of CHX patient bathing.

Methods: 104 freezer-banked *S. aureus* bloodstream isolates, recovered from patients hospitalized for greater than 72 hours, were selected for analysis. Four time periods were studied: (A) pre-CHX patient bathing (before 2009); (B) widespread use of CHX bathing during an institution-wide study (Feb 2009-Aug 2010); (C) washout period with no use of CHX bathing (Sep 2010-Sep 2011); and (D) reintroduction of institution-wide CHX patient bathing (Oct 2011-May 2015). CHX susceptibility was determined by broth microdilution. The Kruskal-Wallis test was used to compare the distribution of MIC between periods; the Mann-Whitney test with Bonferroni adjustment was used for pairwise comparisons between time periods. Isolates were also screened via PCR for the presence of *qacA/B* efflux pump-encoding genes known to mediate reduced susceptibility to CHX in *S. aureus*.

Results: Table 1 shows the number and percentage of isolates per MIC breakpoint. The mean MIC was significantly higher prior to widespread institutional use of CHX bathing compared to the other time periods (p=0.0081): mean MIC ± SD (µg/ml CHX): 0.97 ± 0.46, 0.75 ± 0.57, 0.72 ± 0.26, 0.69 ± 0.25 for time periods A-D, respectively (A vs B, p=0.048, A vs D, p=0.024). Furthermore, no isolates were found to harbor *qacA/B* resistance genes.

Conclusion: Use of an institution-wide CHX patient bathing program for several years has not been associated with decreased susceptibility of *Staphylococcus aureus* to the antiseptic. These findings support continued use of CHX as a safe and efficacious means of reducing nosocomial infections.

Introduction

Hospital acquired infections impact as many as 4% of all patients admitted to acute care facilities[1]

Daily bathing using a solution containing chlorhexidine (CHX), commonly formulated as chlorhexidine gluconate, has shown benefits in reducing the incidence of nosocomial infections:

- Reduced acquisition of multi-drug resistant bacteria such as MRSA and VRE[2]
- Prevention of central line-associated bloodstream infections (CLA-BSI) and decreased bacteriuria[3,4]
- Potential reduction of hospital-acquired *Clostridium difficile*-associated diarrhea[5]

Unfortunately, CHX use may promote the emergence of resistance:

- In Taiwan, MRSA isolates with elevated CHX MIC (>4 µg/ml) increased from 1.7% to 46.7% from 1990 to 2005.[6]
- Patients receiving daily bathing with CHX are more likely to have infecting organisms (MRSA and others) with reduced chlorhexidine susceptibility versus those not receiving such bathing.[7]

qacA/B are plasmid-borne genes that encode for efflux pumps that extrude CHX and other biocides. In staphylococci, the presence of *qacA/B* has been associated with reduced CHX susceptibility and MRSA decolonization protocol failures.[8]

- In Asia, as many as 44% of MRSA isolates have been found to harbor *qacA/B,* versus 0.9% of clinical MRSA samples submitted to a United States surveillance network.[9]

Methods

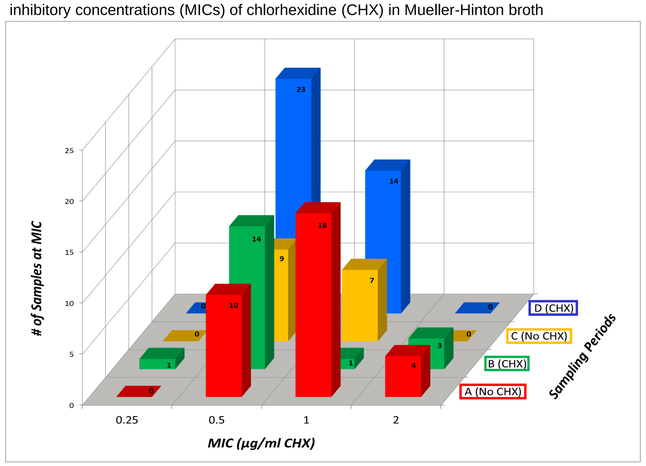
- Purpose of Study: To determine if widespread use of CHX was associated with decreased susceptibility of *S. aureus* to CHX. Bloodstream-infecting bacteria were selected from periods prior to CHX-bathing (Period A), during study use (Period B), during post-study non-use/washout (Period C), and after CHX-bathing protocol adoption/routine use (Period D).
- The null hypotheses were that neither overall susceptibility to chlorhexidine, nor *qacA/B* gene prevalence, would change during or after trial and adoption of chlorhexidine bathing.
- Hospital-acquired *S. aureus* bacteremia was defined by symptom onset and positive blood sample occurring more than 72 hours after admission, to exclude community-acquired infections.
  - 104 banked blood samples, frozen at -70C, were used from Nebraska Medical Center in-patients with *Staphylococcus* bacteremia

Period	Sampling Dates	# Accepted	# Recovered
Baseline (Group A)	12/2007 - 12/2008	39	32
Widespread CHX use (Group B)	02/2010 - 08/2010	20	19
Washout (Group C)	03/2011 - 09/2011	24	16
Widespread CHX use (Group D)	07/2014 - 05/2015	39	37
Total		122	104

- Susceptibility testing of bacteria isolated from blood samples was performed via Mueller-Hinton broth dilutions to determine minimum inhibitory concentration (MIC) of CHX.
- 22 isolates (the 13 with highest MIC, and 9 random others) were also screened for presence of *qacA/B* genes, thought to increase resistance to CHX. Total DNA was isolated, including a *qacA/B* positive control, and amplified via PCR using primers based on NCBI Referece Sequences pSA1379 (qacA) and pTZ2162 (qacB).

Results

Figure 1 – Number of isolates in each period that showed growth at respective minimum inhibitory concentrations (MICs) of chlorhexidine (CHX) in Mueller-Hinton broth



Period	Isolates	Mean MIC	Std Dev	Median	Minimum	Maximum
A	32	0.97	0.46	1.00	0.50	2.00
B	19	0.75	0.57	0.50	0.25	2.00
C	16	0.72	0.26	0.50	0.50	1.00
D	37	0.69	0.25	0.50	0.50	1.00

Table 1 – Statistical distributions of isolates within each time period: mean, standard deviation, median, minimum, and maximum Minimum Inhibitory Concentrations, in µg/ml.

Results

Of 122 patients with nosocomial *S. aureus* bacteremia, 104 had samples available for testing.

Broth microdilution MIC (see *Figure 1)*:

- Maximum MIC was 2 µg/ml chlorhexidine, for 4 isolates in the baseline (period A) and 3 in the study (period B) cohorts.
- MIC_{90} was 1 µg/ml in all time periods.
- There was a statistically significant difference in the distribution of minimum inhibitory concentrations across the four periods (p=0.0081). Specifically, mean MIC for period A was greater than for period B and period D (p=0.048 and p=0.024, respectively). See *Table 1* for distributions.

Presence of *qacA/B* genes:

- Gel electrophoresis bands representing amplified *qacA/B* genes (642 bp) were present only for the positive control (*qac* positive MRSA). PCR analysis demonstrated that none of the 22 isolates tested carried the *qacA/B* genes known to enhance resistance to chlorhexidine.

Conclusion & Future Direction

Bloodstream-infecting *S. aureus strains* have not developed increased resistance to CHX over a 6 year time span including 2 distinct periods with widespread use of CHX. Indeed, a decline in average MIC was seen, and was significant when comparing control (period A) to washout (C) and implementation (D) periods.

No *qacA* or *qacB*-possessing organisms were discovered in the 22 screened strains with the highest CHX MIC.

Limitations:

- Sample size
- MSSA + MRSA inclusion - MSSA carriage of *qacA/B* may be lower than that of MRSA (3.3% vs. 43.8% in one study[10])
- Unrecoverable samples (refrigeration failure)

This study reassures that widespread institutional use of CHX for patient bathing over a period of several years has not been associated with the emergence of *S. aureus* with reduced susceptibility to CHX, and supports CHX patient bathing as a method to reduce healthcare-associated infections and transmission of multi-drug resistant organisms. However, continued monitoring of chlorhexidine MICs is warranted to detect emergence of CHX resistance.

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